

Applicant : Murphy, et al.
Serial No. : 09/619,032
Filed : July 19, 2000
Page : 2 of 15

Attorney's Docket No.: 56446-20003.12/
-004004 / D1120-3

Amendment to the Claims:

Please amend the claims as follows:

This listing of claims will replace all prior versions, and listing, of claims in the application:

Listing of Claims:

Claim 1 (currently amended): A method for hydrolyzing α -glycosidic bonds capable of being hydrolyzed by a polypeptide having an α -galactosidase activity comprising:
contacting a compound having the α -glycosidic bond with an effective amount of a polypeptide having at least a 70% amino acid sequence identity to an amino acid sequence set forth in SEQ ID NO:4 and having α -galactosidase activity.

Claim 2 (currently amended): The method according to claim 1 wherein the polypeptide has at least 90% amino acid sequence identity.

Claim 3 (previously presented): A method of hydrolyzing α -glycosidic bonds comprising:
contacting a compound having the α -glycosidic bond with an effective amount of a polypeptide, wherein the polypeptide comprises a sequence having at least 30 amino acids identical to a contiguous region of amino acids 1 to 364 of SEQ ID NO:4 and has α -galactosidase activity.

Claim 4 (previously presented): The method according to claim 1 wherein the polypeptide has the amino acid sequence as set forth in SEQ ID NO: 4.

Claim 5 (previously presented): The method according to claim 1 wherein the polypeptide is recombinantly produced.

Claim 6 (previously presented): The method according to claim 1 wherein the compound having the α -glycosidic bond is raffinose.

sd-202834

Applicant : Murphy, et al.
Serial No. : 09/619,032
Filed : July 19, 2000
Page : 3 of 15

Attorney's Docket No.: 56446-20003.12/
-004004 / D1120-3

Claim 7 (previously presented): The method according to claim 6 wherein the raffinose is in raw beet sugar.

Claim 8 (previously presented): The method according to claim 1 wherein the compound comprises raffinose, stachyose, verbascose, or a combination thereof.

Claim 9 (previously presented): The method according to claim 8 wherein the compound is contained in a member of the lentil or bean family, or both.

Claim 10 (original): The method according to claim 1 wherein the contacting is at a temperature of about 85°C.

Claim 11 (original): The method according to claim 1 wherein the contacting is at a pH of about 9.5.

Claim 12 (original): The method according to claim 1 wherein the contacting is at a temperature of about 85°C and a pH of about 9.5.

Claim 13 (previously presented): The method according to claim 1 wherein the α -glycosidic bond is an α -1,6 galactosyl bond or an α -1,6 galactosidic bond.

Claim 14 (previously presented): The method according to claim 1 wherein the polypeptide has at least 95% amino acid identity.

Claim 15 (previously presented): The method according to claim 3 wherein the polypeptide comprises a sequence having at least 50 amino acids identical to a contiguous region of amino acids 1 to 364 of SEQ ID NO:4.

Claim 16 (previously presented): The method according to claim 1 wherein the polypeptide has at least 97% amino acid identity.

sd-202834

Applicant : Murphy, et al.
Serial No. : 09/619,032
Filed : July 19, 2000
Page : 4 of 15

Attorney's Docket No.: 56446-20003.12/
-004004 / D1120-3

Claim 17 (currently amended): A method for hydrolyzing α -glycosidic bonds capable of being hydrolyzed by a polypeptide having an α -galactosidase activity comprising:

(a) providing a polypeptide having an α -galactosidase activity, wherein the polypeptide is encoded by a nucleic acid that hybridizes to a nucleic acid having a sequence as set forth in SEQ ID NO:3, or its complementary strand, and the under conditions comprising hybridization conditions comprise hybridization for 30 minutes at 45°C in a solution comprising 0.9M NaCl, 50 mM NaH₂PO₄, pH 7.0, 5.0 mM Na₂EDTA, 10X of 6X SSC, 5X Denhardt's reagent, 0.5% SDS, 100 μ g denatured salmon sperm DNA, and 25% to 50% formamide at 42°C and wash conditions comprising a wash for 30 minutes at room temperature in a solution comprising 150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na₂EDTA, 0.5% SDS of 6X SSC, 0.5% SDS at 50°C; and

(b) contacting a compound having the α -glycosidic bond with an effective amount of the polypeptide, thereby hydrolyzing α -glycosidic bonds.

Claim 18 (previously presented): A method for hydrolyzing α -glycosidic bonds capable of being hydrolyzed by a polypeptide having an α -galactosidase activity comprising:

(a) providing a polypeptide having an α -galactosidase activity, wherein the polypeptide comprises a sequence as set forth in SEQ ID NO:4 or a sequence as set forth in SEQ ID NO:4 having one or more conservative amino acid substitutions, wherein a conservative amino acid substitution comprises substitution of one hydrophobic amino acid for another, or comprises substitution of one polar amino acid for another, or comprises substitution of arginine for lysine, glutamic acid for aspartic acid or glutamine for asparagine; and

(b) contacting a compound having the α -glycosidic bond with an effective amount of the polypeptide, thereby hydrolyzing α -glycosidic bonds.

Claim 19 (currently amended): The method of claim [[17]] 18, wherein the hydrophobic amino acid comprises an isoleucine, a valine, a leucine or a methionine.

Applicant : Murphy, et al.
Serial No. : 09/619,032
Filed : July 19, 2000
Page : 5 of 15

Attorney's Docket No.: 56446-20003.12/
-004004 / D1120-3

Claim 20 (currently amended): A method for hydrolyzing α -glycosidic bonds capable of being hydrolyzed by a polypeptide having an α -galactosidase activity comprising:

(a) providing a polypeptide having an α -galactosidase activity, wherein the polypeptide comprises at least 30 amino acids of a polypeptide portion of a sequence having at least 70% amino acid sequence identity to the polypeptide having a an-amino-acid sequence as set forth in SEQ ID NO:4; and

(b) contacting a compound having the α -glycosidic bond with an effective amount of the polypeptide.

Claim 21 (currently amended): The method of claim 20, wherein the polypeptide comprises at least 50 amino acids of a polypeptide portion of a sequence having at least 70% amino acid sequence identity to the polypeptide having a an-amino-acid sequence as set forth in SEQ ID NO:4.

Claim 22 (currently amended): The method of claim 21, wherein the polypeptide comprises at least 80% amino acid sequence identity.

Claim 23 (currently amended): The method of claim 22, wherein the polypeptide comprises at least 90% amino acid sequence identity.

Claim 24 (currently amended): The method of claim 23, wherein the polypeptide comprises at least 95% amino acid sequence identity.

Claim 25 (currently amended): The method according to claim 1 wherein the polypeptide has at least 80% amino acid sequence identity.

Claim 26 (previously presented): The method of claim 1, claim 17, claim 18, or claim 20, wherein the compound is a food.

sd-202834

Applicant : Murphy, et al.
Serial No. : 09/619,032
Filed : July 19, 2000
Page : 6 of 15

Attorney's Docket No.: 56446-20003.12/
-004004 / D1120-3

Claim 27 (previously presented): The method of claim 17, claim 18, or claim 20, wherein the α -glycosidic bond is an α -1,6 galactosyl bond or an α -1,6 galactosidic bond.

Claim 28 (new): The method of claim 1, wherein the compound having the α -glycosidic bond comprises a saccharide and polypeptide is contacted with the saccharide under conditions which facilitate the hydrolysis of the saccharides, thereby catalyzing the hydrolysis of saccharides.

Claim 29 (new): The method of claim 28, wherein the saccharide comprises a polysaccharide or an oligosaccharide.

Claim 30 (new): The method of claim 29, wherein the polysaccharide or oligosaccharide further comprises a legume.

Claim 31 (new): The method of claim 29, wherein the polysaccharide or oligosaccharide comprises a raffinose, a stachyose or a verbascose.

Claim 32 (new): A method for hydrolyzing α -glycosidic bonds in a compound comprising a raffinose, a stachyose or a verbascose comprising contacting the compound with an effective amount of a polypeptide having at least a 70% amino acid identity to an amino acid sequence set forth in SEQ ID NO:4 and having α -galactosidase activity.

Claim 33 (new): A method for hydrolyzing α -glycosidic bonds in a compound comprising a raffinose, a stachyose or a verbascose comprising contacting the compound with an effective amount of a polypeptide having a sequence set forth in SEQ ID NO:4 and having α -galactosidase activity.

sd-202834